Nuclear Basic Proteins from the Sperm of Tunicates, Cephalochordates, Agnathans and Fish

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ABSTRACT

In this chapter we try to review and arrange the studies on sperm nuclear basic proteins (SNBPs) carried out in several groups of deuterostomes (tunicates, cephalochordates, agnathans, chondrichthyans and osteichthyans). Four general points arise: 1. There are two main types of SNBPs: a. proteins similar to histones but with enhanced basicity, named here "PL" (protamine-like) and b. very specialized proteins, named here "P"; 2. The"PL" proteins have appeared independently several times during deuterostome evolution; 3. In some cases, "P" proteins may have arisen from "PL" proteins, but other origins can not be ruled out for a particular P protein; 4. The classical evolutionary point of view about the appearance of protamines (histones → intermediate proteins → protamines) is re-interpreted as histones → PL proteins → P proteins in this paper. This transition seems to have repeatedly occurred during the evolution of different groups of deuterostomes. Nevertheless, it should not be interpreted as a continuous evolutionary line of the sperm proteins of the whole deuterostome line. In other words, there does not exist an apparent continuous evolutionary line relating the SNBPs of echinoderms with the bony fish and tetrapod protamines.

RÉSUMÉ

Les protéines nucléaires basiques des spermatozoïdes chez les Tuniciers, Céphalocordés, Agnathes et Poissons

Nous essayons dans ce chapitre de faire une synthèse des études concernant les protéines nucléaires basiques des spermatozoïdes (PNBS) qui ont été effectuées sur plusieurs groupes de Deutérostomiens (Tuniciers, Céphalocordés, Agnathes, Chondrichthyens et Ostéichthyens). Quatre points généraux émergent: 1. Il existe deux types principaux de PNBS: a. des protéines similaires aux histones mais avec une basicité augmentée, nommée ici "PL" (proches des protamines), et b. des protéines très spécialisées, nommées ici "P". 2. Les protéines "PL" sont apparues indépendamment plusieurs fois pendant l'évolution des Deutérostomiens. 3. Dans certains cas, les protéines "P" peuvent être apparues à partir des protéines "PL", mais d'autres origines ne sont pas à exclure dans le cas de certaines protéines P. 4. Le point de vue évolutif classique concernant l'apparition des protamines (histones → protéines intermédiaires → protamines) est ré-interprété dans cet article comme: histones → protéines PL → protéines P. Cette transition semble s'être produite de manière répétée pendant l'évolution des différents groupes de Deutérostomiens. Néanmoins, elle ne doit pas être interprétée comme une ligne d'évolution continue des protéines des spermatozoïdes dans l'ensemble de la lignée des Deutérostomiens. En d'autre termes, il n'existe pas de ligne d'évolution continue reliant les PNBS des Échinodermes aux protamines des Poissons osseux et des Tétrapodes.

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In recent years, we have perfomed a series of studies on the SNBPs in deuterostomes intended to link information already available from echinoderms [44], and from several groups of fishes and tetrapods [reviewed in 22, 31-32]. The sperm nuclei of echinoderms contain somatic histones or specific histones with specialized N- and C-terminal domains. In contrast, the SNBPs from fish (also referred to as "typical protamines") are very short proteins (about 30 amino acid residues), consisting mainly of arginine and a few other types of amino acid residues [31]. Two hypotheses have been proposed for the evolution of SNBP in deuterostomes. One of them relates the SNBPs from echinoderms to fish protamines [44]. The second one proposes a foreign origin for the "typical protamine" of bony fish [21, 27]. However, there was a complete absence of information about the SNBPs of deuterostomes from intermediate taxonomic groups. In this chapter we arrange the information already available on the SNBPs of these groups, which may help to clarify some basic and general aspects of the evolution of these molecules. Yet there are several major difficulties in establishing the evolutionary link amongst different SNBPs. They stem from the remarkable variability of these proteins, the limited amount of information still available and the lack of a general consensus about the phylogenetic relationship among different groups of deuterostomes [12, 45].

RESULTS

SNBPs from Tunicates

In recent years, we have studied the SNBPs of several species from four different families of ascidiacean tunicates [9-10, 36]. The main conclusion from these studies is the general constancy in the SNBP pattern exhibited in each of them. Figure 1A shows the electrophoretic patterns of four species, each one belonging to a different family. In all cases, they consist of a major protein (PLasc) (asc = ascidiacean) which migrates close to the position corresponding to histone H4. Each one of the patterns shown in Fig. 1 is representative of all the studied species belonging to the same family, except in the case of the family Styelidae. Within this family, the two species studied of the genus *Styela (S. plicata* and *S. montereyensis)* display a slightly more complex SNBP electrophoretic pattern (Fig. 1B). *Styela* species contain, in addition to the PLasc protein, a significant amount of other proteins.

The PLasc proteins consist of about 150 amino acid residues, and are rich in arginine, lysine, and relatively rich in glycine (Table 1). The studies carried out to date [35, 36] indicate that PLasc exhibits the same tripartite structural organization which is characteristic of histone H1 [19]: An N- and a C-terminal tail flanking a central hydrophobic core (Fig. 1C). The N-terminal region has only two arginine residues (NH_2-R-R-). The trypsin-resistant core consists of 74 amino acid residues and has an amino acid composition (Table 1) and sequence [35] that ressemble those of the trypsin-resistant cores from both H1 histones [1] and some of the SNBPs from bivalves [3] and gastropod molluscs [15]. The C-terminal tail is very basic (Table 1) and its sequence does not display any similarity with the N- or C-terminal tails of the SNBPs from echinoderms ([35] and unpublished results).

In the case of the genus *Styela*, the slow migrating protein (PLsty.1 in Fig. 1B) contains

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NOTE:

In this article the PL proteins of small molecular weight resulting from post-translational cleavage of larger PL precursors have been generically referred to as P proteins. This nomenclature has been adopted to emphasize the structural similarity existing between these proteins and protamines as discussed in the preceding article (AUSIO, this volume).

ABBREVIATIONS:

aa = amino acids, AUT = Acetic Acid/ Urea/Triton Gel Electrophoresis, SDS = Sodium Dodecylsulfate, SNBPs = Sperm Nuclear Basic Proteins

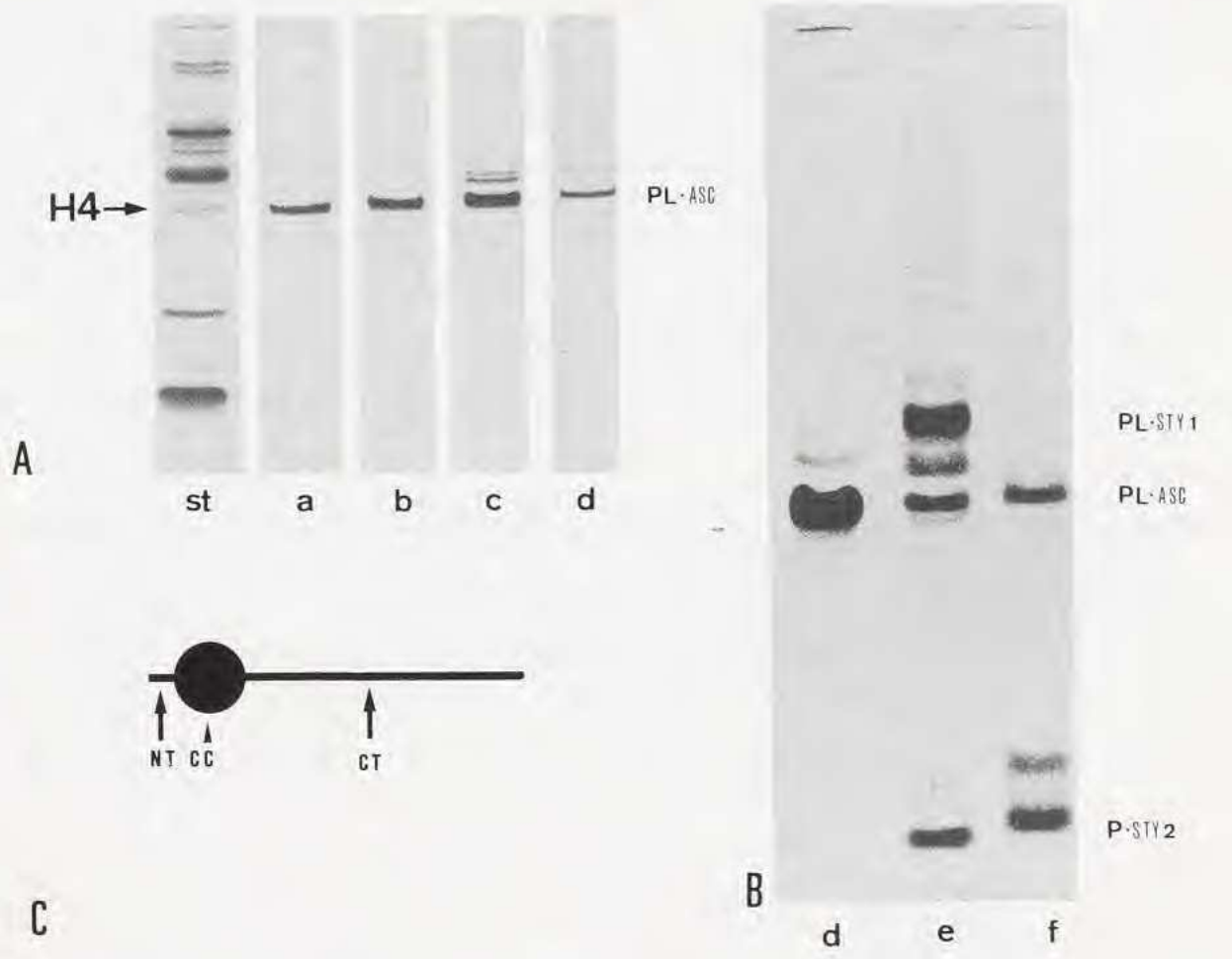

FIG. 1. — **A:** Electrophoretic pattern of SNBPs from four ascidiacean tunicate species. Each species belongs to a different family: a, *Ascidia callosa* (Ascidiidae); b, *Chelyosoma productum* (Corellidae); c, *Boltenia villosa* (Pyuridae); d, *Cnemidocarpa finmarkiensis* (Styelidae); st, proteins from the sperm of the ratfish *(Hydrolagus colliei)* used as a marker standard [10]. **B:** Comparative electrophoretic analysis of PLasc (lane d) and the SNBPs of two species from the genus *Styela*; e, *S. plicata*, f, *S. montereyensis*. The direction of electrophoresis is from top (+) to bottom (-). **C:** Schematic representation of the structural organization of the PLasc molecule; NT: amino-terminal domain (2 arginine residues); CC, trypsin-resistant hydrophobic core (~75 amino acid residues); CT, carboxy-terminal domain (70-75 amino acid residues).

also a trypsin-resistant peptide and an amino acid composition similar to that of PLasc (Table 1) [35, 36]. The protein Psty.2, of higher electrophoretic mobility, displays microheterogeneity and consists of three distinct forms that can be resolved by HPLC [36] and that exhibit an almost identical amino acid composition (Table 1). Table 1 also shows the enormous compositional resemblance that exists between the C-terminal tail of PLasc and protein Psty.2. This fact is of a special interest because it suggests a close relationship between these two molecules (see discussion).

In summary, the sperm nuclei of the ascidiacean tunicates consist of a protein (PLasc) which is different both from specific sperm histones of echinoderm and from the "typical protamines" of the bony fish. This protein appears in all families studied and may represent the

TABLE 1. — Amino acid composition (mol %) of the SNBPs from tunicates (a-g) and from the cephalochordate *Branchiostoma floridae* (h). As a comparison, the composition of a PL III protein (a SNBP from the bivalve mollusc *Crenomytilus grayanus* [29]) is shown in lane (i); a, protein PLsty.1 (PL of *Styela plicata*); b, PLasc (*S. plicata*); c, trypsin resistant core of PLasc (*S. plicata*); d, C-terminal domain of PLasc (*S. plicata*); e-g, each one of the three components of Psty.2 protein (*S. plicata*).

	a	b	c	d	e	f	g	h	i
Lys	14.8	14.3	12.5	9.1	15.8	16.9	17.4	24.7	25.4
His	1.0	1.1	2.1	-	-	-	-	-	-
Arg	33.8	32.5	11.5	58.2	50.8	49.5	50.4	25.3	26.5
Asx	5.1	4.6	8.9	-	0.2	-	1.2	-	0.9
Thr	2.8	1.2	1.2	1.8	2.0	3.1	3.1	-	3.4
Ser	5.3	4.6	8.1	-	1.4	1.4	1.1	16.5	15.6
Glx	2.6	2.2	1.4	3.6	0.3	0.7	-	-	0.9
Pro	2.5	1.4	3.1	-	-	-	-	5.6	5.1
Gly	12.0	13.2	8.1	20.0	21.5	20.8	18.7	6.1	6.5
Ala	8.1	7.1	10.2	3.6	4.5	3.4	3.3	21.7	12.9
1/2 Cys	-	0.7	2.5	-	-	-	-	-	-
Val	4.2	4.6	9.1	-	0.2	-	-	-	1.5
Met	-	1.4	3.3	-	1.3	0.7	0.7	-	-
Ile	1.5	2.4	2.3	-	-	-	-	-	0.4
Leu	2.7	4.5	6.8	3.6	1.9	2.5	3.3	-	0.6
Tyr	2.3	2.2	4.5	-	-	0.9	0.7	-	0.1
Phe	1.3	2.2	1.3	-	-	-	-	-	0.1

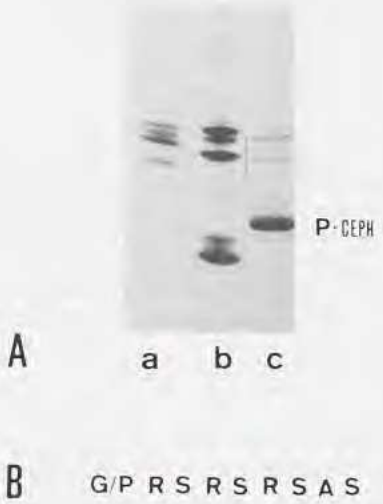

FIG. 2. — **A:** Electrophoretic pattern of *Branchiostoma floridae* SNBPs (lane c), shown in comparison with the SNBPs from the tunicate *Styela plicata* (lane b) and a chicken erythrocyte histones standard (lane a). **B:** First nine amino acid residues (N-terminus) of Pceph protein.

ancestral SNBP of ascidiacean tunicates. Nevertheless, in some particular groups, as in the genus *Styela,* some variations to this general protein pattern have occurred; namely, the appearance of a shorter and more basic molecule (Psty.2) in addition to molecules similar to PLasc (for example, PLsty.1).

SNBPs from Cephalochordates

The phylum Cephalochordata consists of two genera (*Branchiostoma* and *Epigonichtys*) [28]. The SNBPs from *Branchiostoma floridae* have partially been characterized [40]. Their electrophoretic pattern (shown in Fig. 2A) displays a main protein component (Pceph in Fig. 2A) which is accompanied by small quantities of residual histones [40]. Pceph has an electrophoretic mobility similar to that of tunicate protein Psty.2. Its size, estimated from the electrophoretic behaviour is of about 120 amino acid residues, and its compositional analysis (Table 1) is very simple. The protein consists of only six different types of amino acid residues, with arginine, lysine, alanine and serine being the most abundant. It is interesting to note, with regard to the amino acid composition and size, that Pceph is more similar to SNBPs from bivalve molluscs than to other deuterostome SNBPs (Table 1). The N-terminal sequence (shown in Fig. 2B) contains three alternating arginine-serine motives. Such an alternation of basic (R/K) and phosphorylatable residues (S/T) has also been observed in the SNBPs from several gastropod and bivalve molluscs [6, 13, 17], birds and mammals [30-31], but it is not present in the "typical protamines" from bony fish (Fig. 5B).

In summary, the protein Pceph shares some characteristics with typical protamines such as its low amino acid diversity, its basic composition and the presence of serine. Yet, Pceph and "typical" protamines have several distinctive features. First, in Pceph lysine and arginine are present in similar proportions (24.7% and 25.3% respectively) whereas protamines consist almost exclusively of arginine. Secondly, Pceph displays an alanine rich amino acid composition (21.7%) not found in protamines. Finally, Pceph has a much larger size than "typical protamines" and contains the repetitive motifs arg-ser which is absent in bony fish protamines.

TABLE 2. — Amino acid composition (mol %) of the sperm histones of the lamprey *Petromyzon marinus* (PM) in comparison to the somatic histones from calf thymus (CT) [26].

	H1		H2A		H2B		H3		H4	
	PM	CT	PM	CT	PM	CT	PM	CT	PM	CT
Lys	27.6	26.8	9.7	10.2	14.1	14.1	8.3	10.0	10.4	11.4
His	-	-	1.6	3.1	3.3	2.3	1.7	1.7	1.8	2.2
Arg	2.2	1.8	9.8	9.4	8.2	6.9	13.8	13.0	13.4	12.8
Asx	3.9	2.5	6.3	6.2	4.8	5.0	4.2	4.2	4.8	5.2
Thr	3.6	5.6	3.2	3.9	6.6	6.4	7.5	6.8	6.7	6.3
Ser	7.3	5.6	5.2	3.4	10.3	10.4	4.5	3.6	2.0	2.2
Glx	4.7	3.7	9.4	9.8	6.4	8.7	12.0	11.6	6.5	6.9
Pro	9.7	9.2	4.1	4.1	6.2	4.9	4.5	4.6	1.4	1.5
Gly	4.0	7.2	11.4	10.8	5.9	5.4	5.6	5.4	16.8	14.9
Ala	21.8	24.3	13.1	12.9	11.6	10.8	13.3	13.3	7.9	7.7
Cys	-	-	-	-	-	-	0.6	1.0	-	-
Val	6.8	5.4	7.2	6.3	6.0	7.5	4.2	4.4	8.1	8.2
Met	-	-	0.5	-	2.1	1.5	0.8	1.1	0.2	1.0
Ile	1.1	1.5	3.7	3.9	5.8	5.1	4.7	5.3	5.5	5.7
Leu	5.3	4.5	11.9	12.4	4.5	4.9	8.9	9.1	8.6	8.2
Tyr	0.8	0.9	2.3	2.2	2.8	4.0	2.2	2.2	3.6	3.8
Phe	1.1	0.9	0.9	0.9	1.6	1.6	3.3	3.1	2.1	2.1

SNBPs from Agnathans

The nuclear sperm proteins from Agnatha have only been studied in one species of lamprey, *Petromyzon marinus* [40]. Nuclei isolated from ripe sperm of *P. marinus* consist only of histones (Fig. 3A-C). According to the chromatographic and electrophoretic behaviour as well as to the compositional amino acid analyses (Table 2), the sperm histones of *P. marinus* do not exhibit any specific characteristics that distinguish them from the somatic type. This is surprising considering that agnathans have appeared relatively late in evolution and it contrasts with the strong tendency of tunicates and cephalochordates to have specialized proteins in their sperm nuclei. It is necessary to study the SNBPs of more agnathans in order to determine whether the absence of specialized nuclear sperm proteins is just a specific characteristic of *P. marinus* or represents the general trend in agnathans. Nevertheless, the absence of specific proteins and the presence of somatic-like histones in the sperm nucleus also occurs in some groups of bony fish (see below).

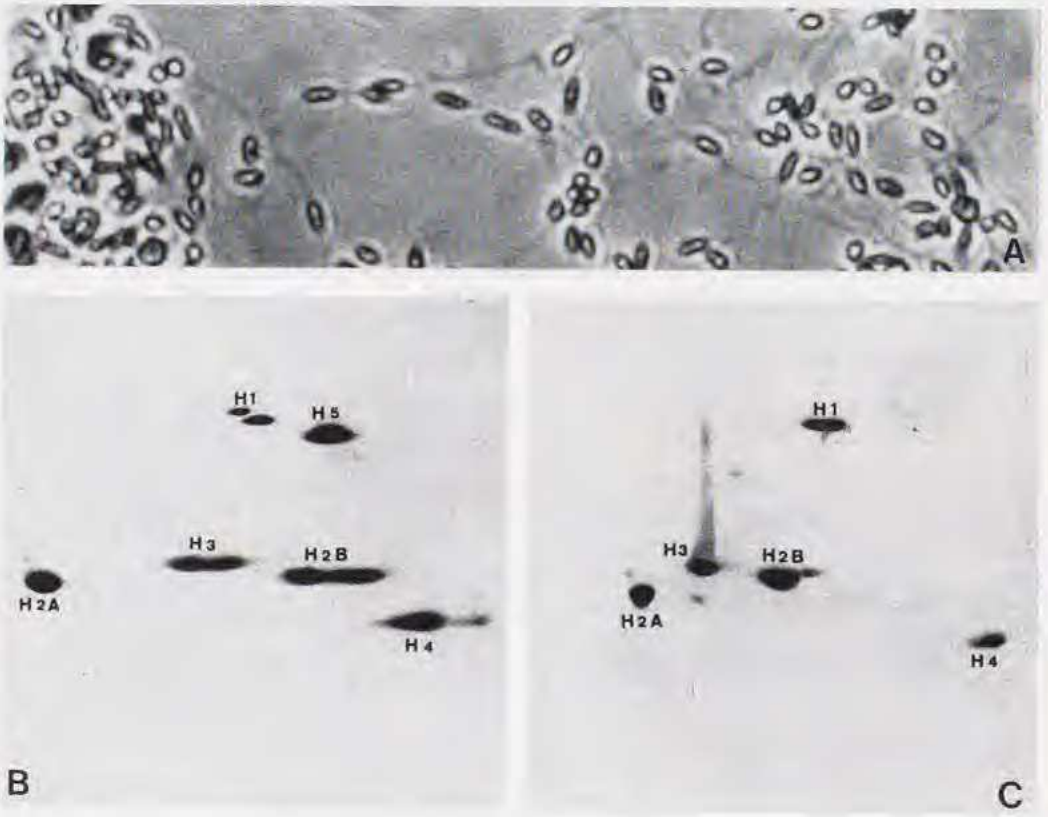

FIG. 3. — **A:** Light microscopy micrograph of ripe sperm used for the analysis of the nuclear sperm proteins of *Petromyzon marinus* (x 1 000). **B, C:** Two-dimensional electrophoretic protein patterns of SNBPs from *Petromyzon marinus* (3C), and histones from chicken erythrocyte (3B) used as standard. The direction of the electrophoresis was left (+) to right (-) (first dimension, AUT) and top (-) to bottom (+) (second dimension, SDS).

SNBPs from Chondrichthyes

The class Chondrichthyes consists of two lineages that diverged very early in its evolution: Holocephali and Elasmobranchii [28]. The Elasmobranchii diverged later giving raise to the Selachimorpha (sharks) and the Batidoidimorpha (skates). The electrophoretic patterns of the SNBPs from eight selachians, one batoidean and one holocephalan species are shown in Fig. 4. All contain several basic proteins in their sperm nuclei. One of these proteins (Pcon.Z3 in Fig. 4) can be directly extracted with dilute acids. However, the other major proteins (Pcon-Z1, Z2) require chemical reduction of the nuclei before they can be solubilized by acids. The need for reduction prior to protein extraction is a characteristic of cysteine-rich proteins owing to the formation of intermolecular S-S bridges in the ripe sperm nuclei. The SNBPs from the selachian *Scyliorhinus canicula* have been characterized previously [18] and they are representative of the SNBP pattern in all selachian species (see the similarity among selachian SNBPs in Fig. 4A). The electrophoretic pattern of the SNBPs of one batoidean species *(Raja rhina)* is shown in Fig. 4A. Unfortunately, there is no information available on the amino acid composition of batoidean SNBPs. The holocephalan *Hydrolagus colliei* contains several minor proteins and three major proteins [37] (see also Fig. 4A). The amino acid compositions of the main *H. colliei* SNBPs are shown in Table 3 in comparison with the corresponding proteins form *S. canicula* .

In Fig. 4B we compare the N-terminal sequence of Pcon-Z3 from *H. colliei* [37] with the

B

(a) ARRRHSMKKKRKSVRRRKTRKNTRKRKNSLGR

(b) ARSRSRRSYGRGRRRGGRRRRRRRRRRRRGGR

FIG. 4. — **A:** Electrophoretic pattern of the SNBPs of eight selachian species (a-h), one batoidean (i) and one chimaeriform (j). a, *Galeus melastomus*; b, *Scyliorhinus canicula* (a, b, family Scyliorhinidae, Order Lamniformes); c, *Centroscymnus coelolepis;* d, *C. crepilater*; e, *Centrophorus uyato*; f, *C. squamosus*; g, *Deania profundorum*; h, *Etmopterus pusillus* (c-h family Squalidae, Order Squaliformes); i, *Raja rhina* (Rajidae, Rajiformes); j, *Hydrolagus colliei* (Chimaeridae, Chimaeriformes). **B:** N-terminal sequence of the protein Pcon-Z3 from *Hydrolagus colliei* (a) compared with the sequence of the protein Pcon-Z3 from *Scyliorhinus canicula* (b) [42].

corresponding protein in *S. canicula* [42] (referred to as Z3 in the original works of GUSSE & CHEVAILLIER [18]).

Judging from the information available, it appears that chondrichthyans share a common SNBP pattern consisting of a very basic cysteine-lacking protein (Pcon-Z3) and a reduced set of cysteine-containing proteins (Pcon-Z1, Z2). It also appears that the amino acid composition and sequences of the SNBPs have undergone a remarkable divergence during the separation of holocephalans and selachians (Table 3 and Fig. 4B; see [37] for a more extensive discussion).

TABLE 3. — Amino acid composition (mol %) of proteins Pcon.Z1, Z2 and Z3 from *Hydrolagus colliei* (HC), and *Scyliorhinus canicula* (SC). Pcon.Z1, Z2 and Z3 compositions from *S. canicula* are from [8, 25, 42]. Res* = Total number of amino acid residues.

	Pcon-Z1		Pcon-Z2		Pcon-Z3	
	HC	SC	HC	SC	HC	SC
Lys	12.2	16.0	9.9	13.0	20.7	-
His	6.9	4.0	1.2	6.5	4.3	-
Arg	30.1	32.0	39.3	37.0	27.6	64.5
Asx	4.3	4.0	1.7	-	4.6	-
Thr	2.8	4.0	4.6	2.2	2.6	-
Ser	4.0	4.0	9.7	4.3	7.4	9.7
Glx	6.0	2.0	1.5	2.2	5.7	-
Pro	6.9	6.0	3.8	2.2	6.9	-
Gly	6.4	2.0	21.1	2.2	4.0	19.4
Ala	2.7	2.0	3.8	10.9	3.4	3.2
1/2 Cys	1.2	8.0	1.4	8.7	-	-
Val	3.5	4.0	-	4.3	1.9	-
Met	1.2	-	-	2.2	1.6	-
Ile	3.9	-	1.7	2.2	-	-
Leu	4.5	8.0	1.8	2.2	3.8	-
Tyr	2.2	2.0	-	-	-	3.2
Phe	1.2	2.0	2.3	-	5.3	-
Res*	79±4	50	66±2	46	45±4	31

SNBPs from Osteichthyes

We have studied a number of bony fish species [35, 38-39, 41] and have exhaustively reviewed the information on osteichthyan SNBPs [41]. The most striking feature that emerged from these analyses was the apparent lack of a unique protein pattern which could be considered representative of all the SNBPs of this group. With a few exceptions [23, 24], the SNBPs of fish belong to one of the five electrophoretic patterns shown in Fig. 5 [39]; namely a, presence of somatic histones and absence of any other sperm-specific nuclear protein (this is a similar situation to that already described in the agnathan *P. marinus*); b, presence of somatic histones but with a marked increase in the histone H1 content (in this case we will consider that the specific nuclear protein corresponds to the additional increase of the corresponding H1); c, presence of a histone complement coexisting with one additional SNBP belonging to PL type; d, presence of one (or few) PL proteins that completely replace histones in sperm nuclei; and e, presence of "typical protamines" which also replace completely somatic histones. There appear to be important differences between "typical protamines" and the SNBPs from groups b, c and d. The former

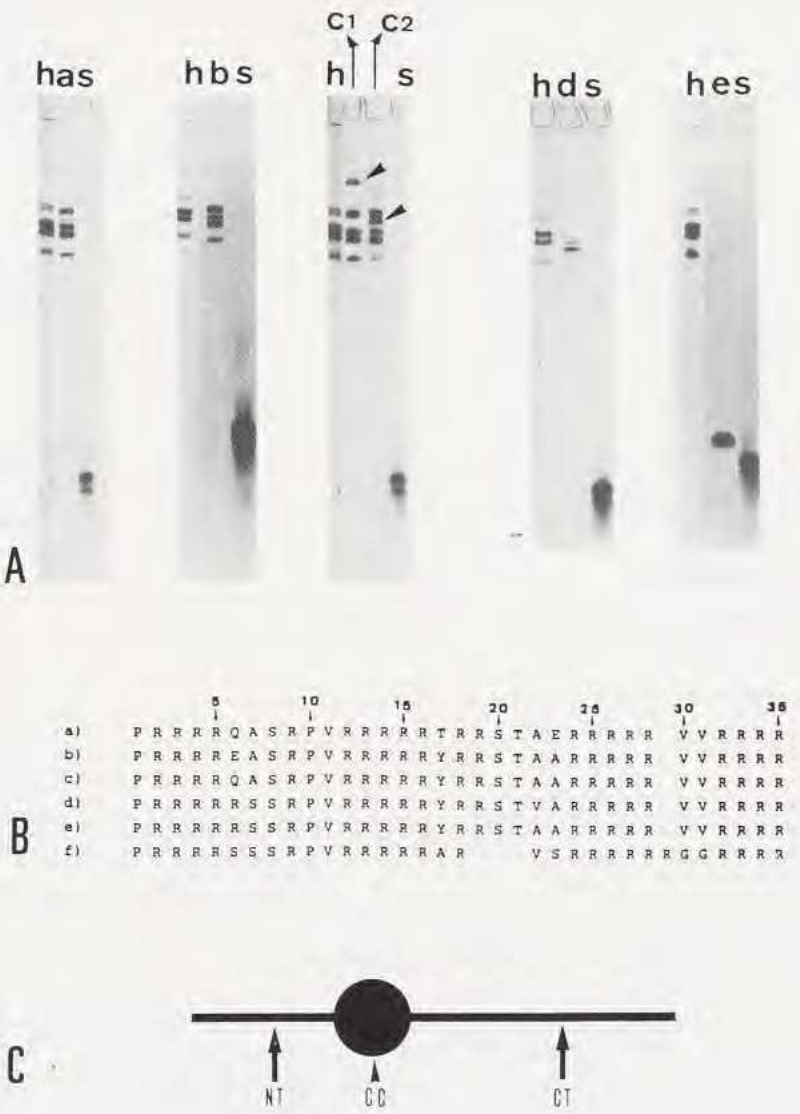

FIG. 5. — **A:** Several representative electrophoretic patterns of the main SNBPs from bony fish; a, somatic histones (*Trigla lucerna*, Triglidae); b, somatic histones with increased quantities of H1 (*Pagellus acarne*, Sparidae); c, somatic histones coexisting with an additional specific protein belonging to PL type (c1: *Merluccius capensis*, Merlucciidae, c2: *Cataetyx laticeps*, Bythitidae); d, protein PL which replaces histones in sperm nuclei (*Mullus surmuletus*, Mullidae); e, "typical" fish protamine (*Dicentrarchus labrax*, Percichthyidae). Chicken erythrocyte histones used as a standard (h) and commercial salmine (s) are shown for comparison in each electrophoresis. **B:** Amino acid sequence of some bony fish "typical" protamines [31, 38]. a, *Dicentrarchus labrax*; b-e, Tuna fish (*Thunnus thynnus*) b, fraction Y1, c, fraction Y2, d, fraction Z1, and e, fraction Z2; f, protamine 2b from rainbow trout (*Salmo irideus*). **C:** Schematic representation of the structural organizaton of PL from *M. surmuletus*; NT: 20 aa residues; CC: ~75 aa residues; CT: 80-85 aa.

are very small molecules consisting of a few amino acid types (see Table 4-e and *D. labrax* protamine sequence [38] in Fig. 5B). In contrast, the SNBP from the other groups are larger molecules, and exhibit a more complex amino acid composition which may be considered similar to that of the histones of the H1/H5-family, but with a higher arginine content (type PL). These

TABLE 4. — Amino acid composition (mol %) of the bony fish SBPs shown in figure 5; a) whole histones from *T. lucerna* sperm nuclei; b) H1 histone of *P. acarne*; c1) specific sperm protein of *M. capensis*; c2) SNBP of *C. laticeps*; d) main PL of *M. surmuletus*; e) "typical" protamine of *D. labrax*.
t = trace amounts

	a	b	c1	c2	d	e
Lys	16.7	21.7	17.9	19.3	24.2	-
His	1.6	-	-	6.5	-	-
Arg	7.9	3.5	19.2	10.4	22.1	61.8
Asx	3.3	4.5	2.0	3.6	5.1	2.9
Thr	4.9	2.9	6.9	5.4	4.5	5.9
Ser	5.0	8.4	6.9	8.4	9.2	5.9
Glx	6.2	6.4	1.3	5.4	t	2.9
Pro	5.7	10.9	14.6	7.8	6.8	5.9
Gly	9.3	11.7	7.7	6.3	5.4	-
Ala	13.4	18.1	14.5	7.1	11.9	5.9
1/2 Cys	t	-	-	-	-	-
Val	7.2	5.4	3.0	4.2	4.9	8.8
Met	t	t	-	3.6	t	-
Ile	5.5	1.1	0.6	3.2	t	-
Leu	9.7	4.6	4.3	6.6	5.8	-
Tyr	1.3	-	1.0	t	t	-
Phe	2.3	0.9	-	1.9	t	-

features are common to both the PL proteins that coexist with histones in the sperm nuclei (lanes b and c in Fig. 5A, and Table 4), and to those PL proteins that wholly replace histones, as occurs in the family Mullidae (Fig. 5A lane d, and Table 4). The electrophoretic pattern of the SNBPs of this family consists of two proteins of almost identical electrophoretic mobility and amino acid composition. They could possibly have arisen from a unique ancestor by a mechanism of gene duplication [38]. The partial sequence of one of them [35] indicates that it consists of approximately 180 amino acid residues organized in three structural domains (N-terminal, central core and C-terminal), as in H1 histones (Fig. 5C). The N-terminal region is 20 amino acid long (50% of them being basic residues) and contains the repetitive motive Ser-Pro-basic-basic which has been described in the N- and C-terminal regions of the sperm histones from echinoderms [33, 43]. The central core is trypsin-resistant and displays a high percentage of similarities with the equivalent region of H1 histones. The C-terminal zone is about 85 residues long and contains most (~70%) of the basic amino acid residues of the molecule.

DISCUSSION

Characteristics and distribution of SNBP molecules

Taking into account the structural and compositional features of the deuterostome SNBPs (other than histones), it is possible to group these molecules into two categories. I) proteins similar to histones (mainly to the H1 type), and II) highly specialized proteins.

The first group, referred to as PL (protamine-like) owing to its enhanced basicity, are relatively large proteins, with a complex amino acid composition similar to that of histone H1. All PL proteins studied to date share the same general molecular organization. All of them have a trypsin-resistant core consisting mainly of neutral amino acid residues, flanked by very arginine and/or lysine-rich N- and C-terminal domains. Depending on the species, PL proteins may be able

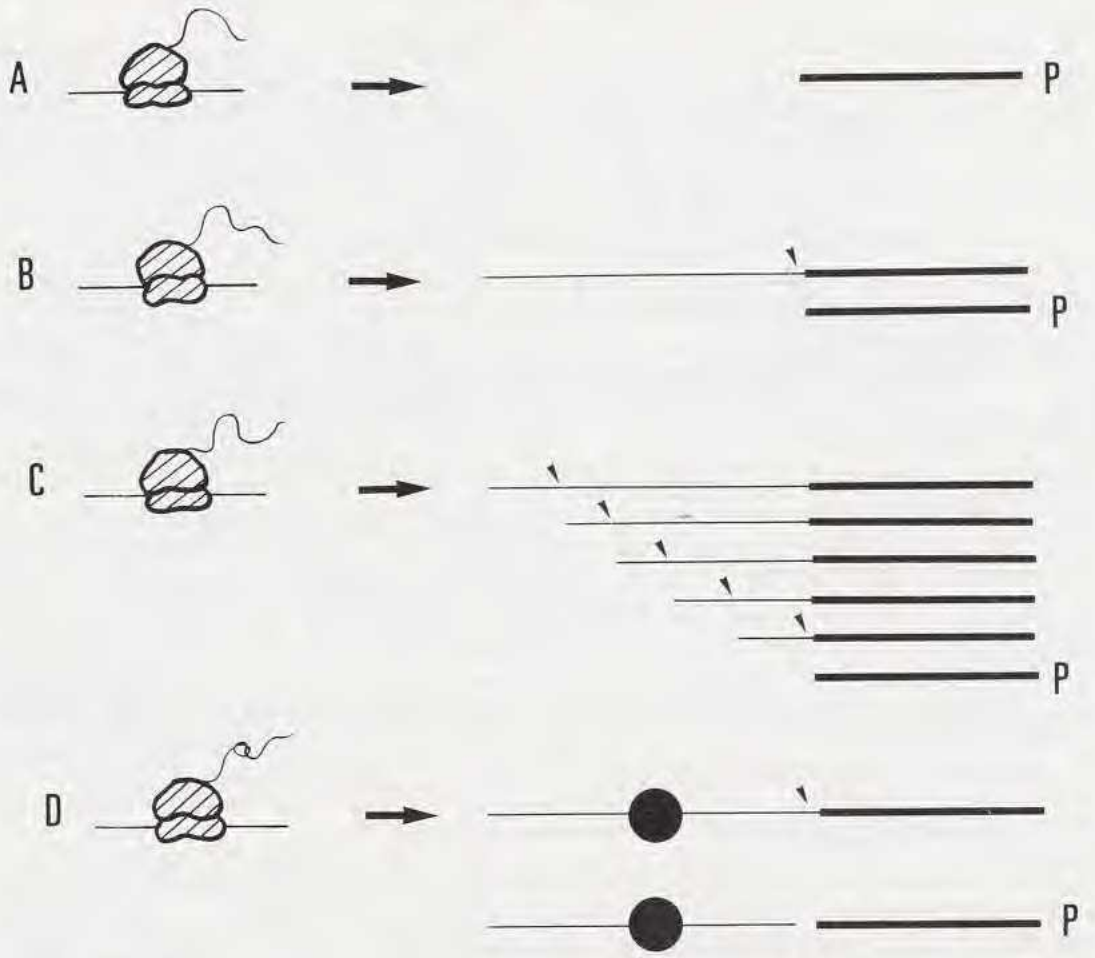

FIG. 6. — Different mechanisms of post-translational processing in SNBPs leading to specialized P proteins: **A:** Direct translation from mRNA; e.g. "typical" protamine of bony fish. [20]. **B:** Translation of a precursor which leads to the appearance of a P protein by a single proteolytic cleavage; e.g. "protamines" of cephalopod molluscs [46]. **C:** Translation of a precursor which undergoes several progressive cleavages. Each cleavage introduces important changes in the extent of chromatin condensation; e.g. "protamines" of neogastropod molluscs [4]; and the mammal P2 protamine [7]. **D:** Translation of a large PL protein. The C-terminal domain is cleaved to generate a very specialized P protein and a second PL protein; e.g. bivalve molluscs [5-6].

to replace somatic histones to different extents. Among the deuterostomes studied so far, PL proteins appear in all ascidiacean tunicates (PLasc) as well as in some fishes (*Mullus, Merluccius*, etc.). However, it is interesting to note that PL proteins are also present in protostomes such as in the case of PL-I and PL-II of bivalve molluscs [3] and "protamines" of patellogastropods and polyplacophorans [15, 16]. The origin of this type of protein is not well established. From the sequences published [3] and the ongoing analysis of the sequences of PLasc and PL from *Mullus* [35], it becomes apparent that the central core of these proteins shares an enormous similarity with the globular core of the histones of the H1 family. Therefore, PL proteins may be evolutionarily related to this histone. In contrast to the central core, the PL N- and C-terminal tails exhibit an enormous compositional variability. From the evolutionary point of view, there does not seem to exist a direct link among the zoological groups which display PL proteins. In our opinion, the PL sperm specific proteins may have appeared independently, several times during the evolution of

the animal kingdom (bivalves, polyplacophors, patellogastropods and tunicates, and several times among fish).

The proteins of the second type, which we refer to here as "very specialized proteins" (or "P" proteins), exhibit the following characteristics: they are short molecules (from approximately 30 to 120 amino acid residues) and they exhibit a very simple amino acid composition (with only a very few amino acid types). Arginine and/or lysine are present in a large amount (50%-80%) and hydrophobic residues are scarce or absent. Among the deuterostomes studied here, P proteins appear sporadically in tunicates (Psty.2 in genus *Styela*), in cephalochordates (Pceph), in chondrichthyan fishes (Pcon.Z3) and in osteichthyes (typical protamines). However, as occurs with PL proteins, P proteins are widely distributed over a broad spectrum of both phylogenetically distant and closely related groups. Thus P proteins have been described in bivalves [2], archaeogastropod molluscs [13-14, 16-17], neogastropods ("ripe" protamines) [4], cephalopod molluscs [46], crustaceans [11], echinoderms (Φ_0 protein) [34] as well as in fish, amphibians, birds and mammals [22, 31, 47]. These P proteins are not always homologous and it is reasonable to think that they have also appeared independently several times and from several different cellular processes (Fig. 6) during the course of evolution. In the case of bivalve molluscs, it has been demonstrated that some "specialized proteins" (PL-IV in the original works of CARLOS *et al.* [5-6]) actually correspond to the C-terminal region of a larger protein precursor belonging to the PL type (referred to as PL-I by some authors) (see also AUSIO, this volume). They appear in the nuclei following a precise proteolytic cleavage (Fig. 6D). From this perspective, the similarity between the amino acid composition of the Psty.2 protein and the C-terminal part of PLasc (Table 1) is very suggestive of a possibly related cleavage post-translational mechanism in the case of these proteins. It seems possible that some P proteins have arisen from a simplification (genotypical or phenotypical) of other proteins of the PL type, although other origins for particular P proteins (as is the case of Pcon.Z1, Z2) can not be disregarded.

General evolutionary considerations

From the information presented in this chapter, it is quite evident that it is difficult to trace an uninterrupted evolutionary line connecting the different SNBPs of deuterostomes. The proposition of an evolutionary link between histones and protamines which has been observed in different phylogenetic groups (histone H1 → PL proteins → very specialized P proteins; see also AUSIO, this volume) has most likely occurred independently in each of them. Another important consideration to be made arises from the fact that when a group consisting of a large number of species is analyzed, the evolutionary trend within this group may be masked by the variability of SNBPs present in the limited sample of species analyzed. This is for instance the case in bony fish where the appearance of "protamines" seems to be sporadic. Two hypothesis have been put forward to explain the apparent random distribution of SNBPs within this group. The first one proposes a mechanism of horizontal evolution of the genes of the fish protamines. In the second alternative, the phenomenon is explained by the loss of the expression of these genes during the formation of some groups of fish [21, 41]. It is important to stress the fact that fish protamines, which for historical reasons have long been considered to be the "typical protamines", in fact represent only one of the many types of the highly specialized P proteins. Fish protamines do not necessarily represent the final goal of SNBP molecular evolution. Yet, they are of special interest because of the extent of similarity they share with the P1 protamine ancestors of tetrapods [31].

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